THE BLOOD OF THE ATLANTIC SALMON DURING MIGRATION

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There are many alterations in the habits and metabolism of salmon during their migration from the sea into the rivers. Among the metabolic changes, one can be well defined in relation to an equally distinct alteration of the environment of the fish. In fresh water the freezing point depression of the blood of salmon is less than it is while they are in salt water (Greene, 1904; Smith, 1932). As a consequence of the reduced salinity of the blood, which is indicated by the reduction of the freezing point depression, it might be expected that the condition of the blood for the transport of oxygen would be affected; for it is known that the affinity of hemoglobin in solution for oxygen is diminished by increasing concentrations of salt (Barcroft and Camis, 1909).

The affinity of the hemoglobin in the blood of several freshwater fish for oxygen is greater than in the blood of some saltwater fish, as is shown by the pressure of oxygen required for half saturation of the blood of a few marine and freshwater species in Table I. The freezing point depression of the blood of freshwater fish is usually less than in saltwater fish, and the blood of fish migrating from salt into fresh water undergoes dilution, as is shown by the examples in Table II. The examples quoted are too few to warrant more than the suggestion of the effect of salinity upon the blood, and there are many influences beside the salinity of the environment which will operate to differentiate the blood of various species.

There is an advantage in examining the blood of individuals of the same species in two environments separated by so short an interval as that which comes between the salmon in the brackish estuary and in the lower fresh water reaches of a stream. We have found it possible to distinguish the conditions for oxygen combination with the blood of Atlantic salmon, *Salmo salar,* caught in salt water from those of the fish which were caught in the rivers. The changes in oxygen affinity occurred as the freezing point was changed by the passage of the fish into fresh water. The changes observed are large enough to suit the blood for respiratory transport under somewhat different conditions, and may be critical in determining respiratory ability in certain natural situations.

Materials and Methods

The blood of the Atlantic salmon, *Salmo salar,* was chosen because of its accessibility. These fish spawn and are hatched in the rivers which drain into the coastal waters of the Province of Quebec, Newfoundland, Nova Scotia, and New Brunswick. They spend the first two to five years of their lives in the rivers and then migrate to the ocean, where they spend one, two, or three years before returning to the rivers to spawn. The major spawning migrations occur in the spring and fall

Table I

A comparison of the tensions of oxygen required for half saturation of the blood of some salt and freshwater fish

Fish	Tension at half saturation mm. O_2	pCO_2 mm.	Temperature °C.
Fresh water			
Bowfin *	4	0–1	15
Amia calva			
Common sucker *	12	0–1	15
Catostomus commersonnii			
Carp *	5	1–2	15
Carpiodes cyprinus			
Pike †	3.5	7.5	18
Esox lucius			
Salt water			
Cod †	15	7.5	14
Gadus callarias			
Sea robin ‡	16	1	20
Prionotus carolinus			
Mackerel ‡	16–17	1	20
Scomber scombrus			
Toadfish ‡	14	1	20
Opsanus tau			

* Black.
† Krogh and Leitch, 1919.
‡ Root, 1931.

of the year, but fish in greater number and of larger size come in the spring (at least in the St. Lawrence region (Belding and Prefontaine, 1938)). Our samples of fish were obtained in the late spring and early summer from the gill nets of commercial fishermen situated around the mouth of the York River, which empties into Gaspé Bay, Province of Quebec. These fish were in brackish water and, unfortunately, no true saltwater fish were obtained. Freshwater fish were had from the lines of the sport fishermen on the St. Jean River, which also empties into the Bay. Two of the freshwater fish were kindly supplied to us from the salmon of the Gaspé Hatchery.

The fish were all two- and three-year "sea life" salmon averaging roughly 10 and 20 pounds in weight respectively. They were bled by heart puncture, 20 to 80 cc. being obtained from a single fish. Heparin was used as the anticoagulant throughout, and the blood was stored on ice from the time of drawing until it was used.

The blood was equilibrated with gas mixtures in the special tonometers designed by Irving and Black (1937). Gas analyses on blood were done by Van Slyke's manometric method. The temperature of equilibration was 15° C. ± 1.0°.

Freezing point determinations were made with a micro-Beckmann thermometer on 2 cc. samples of plasma. Despite the small samples, most duplicate determinations agreed to within .005° C.

Hematocrit determinations of relative erythrocyte volume were made on all specimens in the usual capillary tubes in a centrifuge operating at 5000 r.p.m.

Table II

A comparison of the freezing point depressions of the blood of fresh, salt water and migratory fish. Modified from Smith (1930)

Fish	Freezing Point °C. Medium	Freezing Point °C. Fish plasma
Amia calva	.03	.54
Lepidosteus osteus	.03	.57
Anguilla rostrata	.08	.63
" "	1.85	.82
Conger vulgaris	2.15	1.03
Salmo salar	0	.64
" "	.87	.77

Oxygen Capacity

The oxygen capacities (Table III) of the bloods of 15 brackish water fish varied between 10.5 and 14.9 volumes per cent, with an average capacity of 12.3 volumes per cent. The proportion of erythrocytes in the blood of these fish varied from a minimum of 24.4 per cent cells to a maximum of 47.5 per cent cells, averaging 39.4 per cent. In 6 freshwater fish the oxygen capacities varied between 6.7 and 10.0 volumes per cent with an average of 8.8; the proportion of erythrocytes ranged from 19.3 to 28.4 per cent, averaging 24.8. If we calculate the oxygen capacity of 100 cc. of cells for the two kinds of fish, we find that for the brackish water fish the average value is 31.6 cc., whereas for the freshwater group it is 34.9 cc. It seems from these figures that the proportion of cells in the blood of freshwater fish was decreased without diminution of the oxygen capacity of the cells.

Oxygen Dissociation Curves

Points on the oxygen dissociation curve of the brackish water fish show a considerable scatter. The points are taken from 14 different fish. It can be seen from Fig. 1 that at half saturation the spread is from about 17.5 to 28.8 mm. of O_2 tension. The mean curve has a half saturation at 23 mm. O_2 tension. The curve is similar to those found for other marine fish by Root (1931). The curve shows an interesting tendency to have an " S " shape similar to that of mammalian bloods, and is in most other respects similar to these well-known curves.

In contrast to the brackish water fish, it is to be noted that with the exception of one point the points obtained from 4 freshwater fish fall nicely on a continuous curve. This curve is rather steeper than the others and lies to the extreme left of them with a half saturation at about 19 mm. of O_2 tension (Fig. 1).

Table III

A comparison of the data on brackish and fresh water salmon

	Brackish water fish	Fresh water fish
Cell volume	39.4%	24.8%
Variation	24.4–47.5 (15 fish)	19.3–28.4 (6 fish)
Oxygen capacity	12.3 vols. %	8.8 vols. %
Variation	10.5–14.9 (15 fish)	6.7–10.1 (6 fish)
Maximum CO_2 effect	62.8% Sat.	57.2% Sat.
Variation	56–67 (5 fish)	56–58 (4 fish)
Cell volume increase	9.0%	8.2%
Variation	8.1–13.9 (5 fish)	6.2–11.1 (4 fish)
Depression of freezing point	0.77° C.	0.64° C.
Variation	0.72–0.80 (5 fish)	0.60–0.68 (7 fish)

The Effect of CO_2 upon Oxygenation

Carbon dioxide prevents the saturation of salmon blood with oxygen at 150 mm. oxygenation (Fig. 2). This has been observed in the blood of other marine and freshwater fish (Root, 1931; Black and Irving, 1938; Irving, Black and Safford, 1941).

There is a considerable spread in the points obtained from the brackish water fish, the maximum effect of CO_2 restricting the oxygenation of hemoglobin to between 56 and 67 per cent saturation. In the freshwater fish CO_2 restricted oxygenation to about 58 per cent saturation for the maximum effect. Neither curve for salmon blood seems to flatten out quite as quickly as the curve of carp blood (Black and Irving, 1938), and even beyond pressures of 80 mm. of CO_2 there appears to be some further depression of oxygenation. Hemolysis does not abolish the effect of CO_2 either in the brackish or freshwater fish, and causes a decrease of not over 15 per cent in its magnitude. Hemolysis of trout blood likewise does not much reduce the effect of CO_2 upon oxygenation

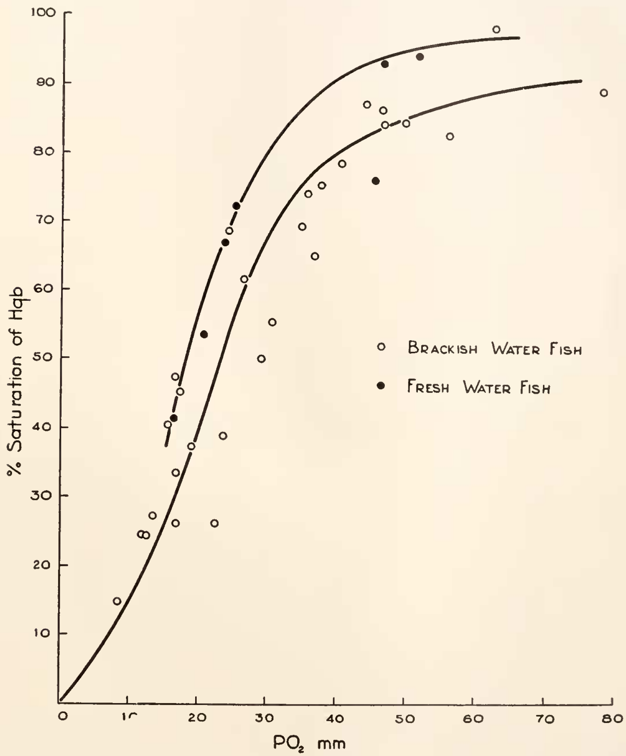

FIG. 1. The O_2 dissociation curve of the blood of salmon taken from brackish and fresh water. P CO_2 less than 1 mm.

(Irving, Black and Safford, 1941). There are evidently two categories of fish blood, in one of which hemolysis abolishes the CO_2 effect (Black and Irving, 1938; Root and Irving, 1940), while in the other hemolysis has little influence. Hemolysis by either freezing and thawing or by the use of saponin produces the same result.

The effect of CO_2 on the oxygen dissociation curve of brackish water fish is shown in Fig. 3. The pressure at half saturation of the hemoglobin is 40 mm. of oxygen when the CO_2 tension is 13–14 mm. compared with the average of 23 mm. O_2 tension with the CO_2 tension of 1 mm. or less. The effect of CO_2 upon the oxygen dissociation curve of freshwater salmon was not determined, but from the maximum effect of CO_2 on the oxygen capacity in fresh and saltwater fish it seems likely

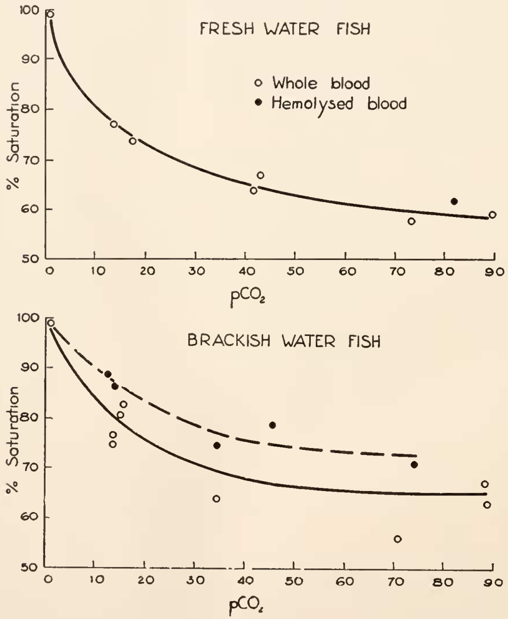

FIG. 2. The effect of CO_2 upon oxygen dissociation curves of salmon blood.

that the effect of CO_2 on oxygen dissociation curves would be similar in salmon from salt and fresh water.

EFFECT OF CO_2 ON CELL VOLUME

Carbon dioxide has a marked effect in causing the cells of salmon blood to swell (Fig. 4), as was shown for the blood of some freshwater fish by Black and Irving (1938), and for the blood of trout (Irving, Black and Safford, 1941). The magnitude of the effect on the cell volume in brackish water fish averaged 9.0 volumes per cent of cells, with a variation of 5.1 to 13.9 volumes per cent in 5 fish. In the freshwater fish the swelling averaged 8.2 with a variation of 6.2 to 11.1 in 4 fish. The reversal of the effect on complete oxygenation of the blood without CO_2 is demonstrable.

Most of the swelling is produced at low tensions of CO_2, and it indicates some 25 per cent enlargement of the erythrocytes. The considerable swelling of the cells is of a much greater magnitude than the osmotic changes which are described in mammalian blood, and there seems to be no satisfactory explanation for this phenomenon.

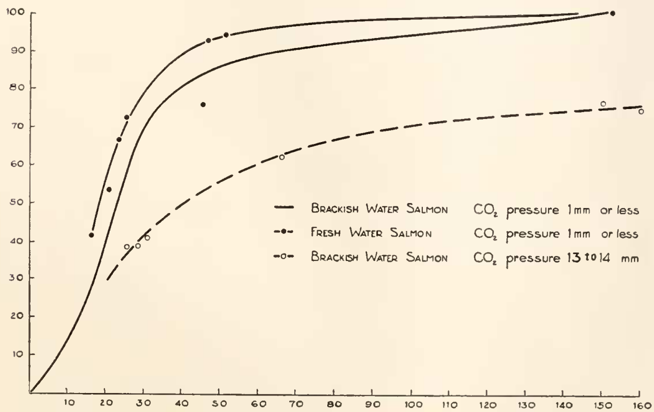

Fig. 3. The maximum effect of CO_2 upon the degree of oxygen saturation at $P\ O_2 = 150$ mm.

The Combination of CO_2 with Blood

In Figure 5 is shown the curve describing the combination of CO_2 with the blood. This curve indicates that in contrast to the reduction in oxygen capacity which occurs in fresh water, there is no significant difference in CO_2 combination.

Freezing Point of the Plasma

The effect of the change of external environment upon the blood plasma is demonstrated in the change in the freezing point depression. In the plasma of brackish water fish freezing points varied from —0.717

to — 0.800° C., with an average value of — 0.765° C. for the 5 bloods examined. In the freshwater fish 7 determinations fell between — 0.597 and — 0.675° C., with an average of — 0.638° C. One other fish

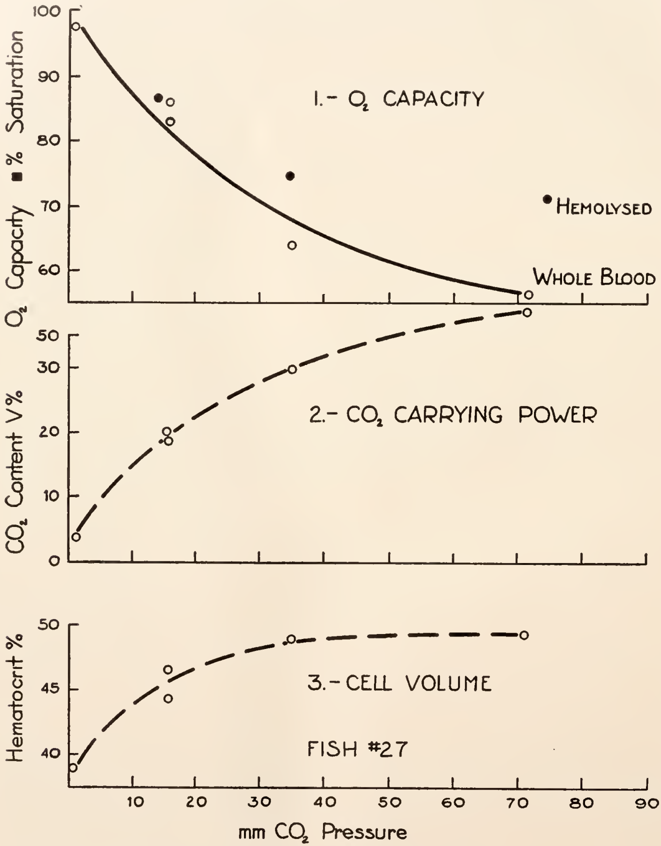

FIG. 4. The effect of CO_2 upon: (1) oxygen saturation, (2) CO_2-carrying power, and (3) cell volume in the blood of one salmon.

showed a value of — 0.775° C. This fish was caught much lower down in the river than any of the others, although still in the fresh water. It was caught at the very end of the season when it is known that the late stragglers spend very little time in becoming acclimated to the brackish water, and this observation may explain the individual discrepancy.

Discussion

We would like primarily to answer the question in this paper of whether or not there is a difference in the properties of the blood of a migratory fish when it is in equilibrium with a marine and freshwater environment, and the changes which we are particularly interested in are those of the system for respiratory transport.

There is undoubtedly a drop in the concentration of cells of the salmon in fresh water, and concomitantly there is a drop in the oxygen capacity of the blood (Table III). There appears to be no difference in the hemoglobin concentration of the cells of the two kinds of fish.

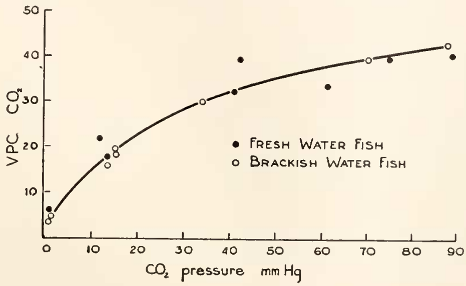

Fig. 5. The combination of CO_2 with salmon blood at P $O_2 = 150$ mm.

Dilution of the serum also takes place when the fish moves into fresh water, as shown by the decrease in the freezing point depression. If we examine the oxygen dissociation curves of the fish in its different habitats, there appears evidence that here too there is a change with migration. The curve for the freshwater salmon lies on the outermost border of the scatter of points belonging to the brackish water inhabitants (Fig. 1), and the points hold well to a smooth curve.

Considering the brackish water fish in relation to their environment, we find: (1) that the environment varied somewhat in its osmotic concentration with respect to the tide and the location in the bay in which they were caught; (2) that the fish had been in this environment of lower salinity than the sea [1] for various lengths of time and were consequently in various stages of acclimitization. In view of this we might

[1] The freezing point depression of a sample of water from the mouth of the York River, at which point many of the fish were taken, was 0.87.

expect to find the variation which was actually observed, and also to predict that the blood of true marine fish should give points lying at the extreme right of the group. We are inclined then to believe that in this important characteristic, the affinity for oxygen, the blood of salmon living in fresh water differs from those taken from salt water.

The provision for transporting oxygen in fresh water is less since the oxygen capacity of blood is only about two-thirds of what it is in the brackish water, and probably less than that in relation to the oxygen capacity of true marine fish. One is inclined to wonder if the fresh-water environment imposes a more sedentary mode of life because of the changes which are brought about in the internal conditions of the salmon.

In affinity for oxygen the blood of fresh water salmon equals that of three freshwater salmonoid fish,—the brook trout, brown trout, and rainbow trout (Irving, Black, and Safford, 1941). In all of these fish atmospheric tensions of oxygen saturate the hemoglobin at 15°. But as the temperature is raised, the affinity of trout blood was found to diminish, until at about 25° atmospheric pressures of oxygen could no longer secure saturation. It is quite likely that a similar temperature effect prevails in salmon blood, which should therefore be suitable for oxygenation at 25° in thoroughly aerated water. The blood of salt-water salmon would probably fail to saturate at about 20°, but it is likely that the well-circulated tidal waters are always well enough aerated for adequate oxygenation. In the warm water of the rivers in summer even the blood of the freshwater salmon encounters temperatures which are near the limit permitting saturation. If the oxygen tension in the warm water is depleted below atmospheric pressure, then the blood cannot become saturated with oxygen in the gills. In the rivers it is likely that stretches of warm water, partcularly if they are not well aerated, act as barriers by hindering the transport of oxygen. Under such conditions the effect of temperature upon oxygen affinity may have a critical influence in determining where the fish can exist.

We see that the plasma of freshwater fish has a lower freezing point than the brackish water fish, and that this is due to a reduction in the electrolyte concentration has been shown by Homer Smith (1930). In our freshwater salmon, the serum has been diluted and we may surmise that in order to reestablish osmotic equilibrium, water has diffused into and perhaps salts out of the cells. That the latter is so would seem to be borne out by the values of oxygen carried by 100 cc. of cells. Long ago Barcroft and Camis (1909) showed that when hemoglobin solutions are dialyzed, the oxygen dissociation curve for the solution, when compared with the curve for the undialyzed solution, is shifted some to the left, and hence saturates at a lower pressure. It should be pointed out that they were working with rather dilute solutions of hemoglobin and also were dialyzing off the last portion of electrolyte and therefore the conditions in the salmon blood are hardly comparable.

It is rather remarkable that the CO_2 dissociation curves for the freshwater and saltwater fish (Fig. 5) should be the same in spite of the difference in oxygen capacity. There are large differences in CO_2 capacity of the blood of different species of fish which appear to be quite unrelated to oxygen capacity.

The changes which have been shown in the blood of migrating salmon are large enough to be important to the economy of respiratory metabolism. The relation of these changes in the blood to the change in environment suggests how the detailed physiology of the salmon changes with the varying environment.

Summary

The blood of Atlantic salmon caught in the brackish water of Gaspé Bay has been compared with the blood of salmon caught in the fresh water of the rivers draining into the Bay. In brackish and fresh water the average properties of the blood are respectively: oxygen capacity, 12.3 and 8.8 volumes per cent; cell volume, 39.4 and 24.8 per cent; oxygen tension for half saturation at T $CO_2 = 1$ mm., 23 and 19 mm.; freezing point of the serum, —0.79 and —0.64. The oxygen combination at P $O_2 = 150$ mm. in the presence of large tensions of CO_2 is reduced to about 60 per cent of saturation. Hemolysis does not much reduce the CO_2 effect. The cells swell greatly as the CO_2 tension is increased. There appears to be a dilution of the blood as the fish goes from salt to fresh water. This is seen in the decrease in cell volume, oxygen capacity, and freezing point depression of the blood. It seems also that in fresh water the affinity of the hemoglobin for oxygen is greater than in salt water. The changes observed in the blood may be related to the change in salinity of the environment. In the warm water of rivers in summer small changes in temperature and oxygen saturation may be critical in determining whether or not the blood can be saturated with oxygen.

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